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4 May 2005

**BY FAX TO: 00-4989-2399-4465**
**FROM: 020-7430 7600 - 11 PAGE(S)**
**CONFIRMATION BY COURIER**

Dear Sirs,

**International Patent Application No. PCT/GB04/002678**
**In the name of: PEPHARM R&D LIMITED**
**Representative's Ref: SCB/MPS/P72471WO00**

In response to the Written Opinion dated 4 March 2005, I enclose amended claims 1 to 8 to replace the claims currently on file. The amended claims are presented as a clean copy and in mark-up form for the assistance of the Examiner.

**Remarks**

With this reply, former claims 1, 5, 9, 11, 17, and 18 are amended and claims 3, 4, 6-8, 10, 13-16 and 19 are cancelled. Accordingly, claims 1, 2, 5, 9, 11, 12, 17 and 18, renumbered as claims 1 to 8, are pending upon entry of these amendments. No new matter has been introduced.

Applicants respectfully request reconsideration and further examination of the application in view of the amendments and arguments presented herein.

**Novelty**

The Examiner has raised Novelty objections against previous claims 1-5, 7, 8 and 19 in view of document D3 and against claims 5 to 8 having regard to document D2. While Applicants respectfully disagree, claims 3, 4, 6-8 and 19 have now been cancelled. Therefore, the objections to these claims are moot.

**1. D3**

Amended claims 1 and 2 are directed to an isolated or purified tripeptide consisting of YSV. The term "isolated" or "purified" clearly and unambiguously defines the claimed invention as an isolated tripeptide that is not attached to any support units, nor is existent in a mixture of hundreds of other tripeptides.

D3 teaches a new spatially addressable split method of preparing a 125-member tripeptide library by using Chiron crowns as solid support units and a manual sorting device (see D3, Page 220). To synthesize the tripeptide library, D3 requires the crowns to go through first coupling, first sorting, second coupling, second sorting and third coupling. Each coupling means each of the crowns would attach one of the amino acids, and each sorting means the crowns carrying attached amino acids were transferred for sorting (see page 222). After the third coupling, tripeptides are formed "on the crowns of strings." (see page 223, left column).

D3 does not disclose isolation or purification of the tripeptide YSV, nor does it specify that the synthesized amino acids are L-form amino acids. D3 teaches a 125-member tripeptide library that comprises a member that is in the form of YSV-*. The YSV-* in D3 is not an isolated or purified tripeptide because the YSV therein is still attached to the Chiron Crown amid the rest of 124 tripeptides-*, all of which are attached to respective, individual Chiron Crowns as well.

Nor does D3 suggest, or provide a motivation of, isolating or purifying or separating the tripepide YSV from the attached Chiron Crown. The only peptides that were isolated were the 5 peptides boxed in Table 3, and this was only done for the purposes of verification (column 1 page 223).

Therefore, D3 is not novelty-destroying, nor is relevant to the inventive step, of claims 1, 2 and 3.

**2. D2**

The Examiner also objected that former claims 5, 7 and 8 lack novelty in view of D2. Claims 7 and 8 have now been cancelled. Therefore, the Examiner's objections to claims 7 and 8 are moot.

The objection raised against claim 5 was due to its juxtaposition to claim 6 and having different terminology, which lead to the suggestion that the scope of claim 5 extends to peptides comprising the tripeptide YSV. While Applicants respectfully disagree, claim 6 has now been

cancelled and claim 5, now renumbered as claim 3, amended to specify a polypeptide "consisting of" the tripeptide L-Tyrosyl- L-Seryl-L-Valine.

D2 discloses the peptide YSVT and a composition thereof. The YSVT in D2 is a tetrapeptide and therefore, cannot fall within the scope of claim 3, which specifies a tripeptide. Therefore, D2 fails to be novelty-destroying for claim 3.

**Inventive Step**

**D1**

The Examiner has objected that former claims 6 and 9-19 lack an inventive step in view of D1. Specifically, the Examiner observes that D1 teaches tripeptides YSL and YSF modulating the immune response and growth of cancers, and that for peptides having non-polar or hydrophobic side chains it may be possible to substitute one side group for another without reducing biological activity. The examiner further alleges that there is not a large number of possible substitutions and thus, this is not considered a selection invention with a surprising technical effect.

Former claims 6, 10, 13-16 and 19 have now been cancelled. Therefore, the Examiner's objection to these claims is moot. The objection to claims 9, 11, 12, 17 and 18, now renumbered as claims 4 to 8, is respectfully traversed. The examiner erred in the analysis of the inventive step in this case for the reasons as follows.

D1 discloses, *inter alia*, the tripeptide YSL. The difference between this particular peptide disclosed in D1 and the present invention is, therefore, that the claimed tripeptide has the peptide sequence YSV.

The technical effect of this difference is that the tripeptide YSV has a number of surprising properties, such as the ability to prevent leukaemia and melanoma growth, which the prior art tripeptide YSL does not have. Thus, the Examiner is incorrect in stating that the tripeptides of D1 have the same technical effect as YSV. Detailed support for the surprising and differing technical effects is provided below (see "II Unexpected technical effects").

The problem to be solved, therefore, contrary to what was alleged by the Examiner, is how to provide an improved peptide which can prevent leukaemia and melanoma growth and also have

improved efficacy with respect to liver cancer. This problem is derivable from the application as filed, see page 3 line 12-13, page 4 line 9 and claims 4 and 17 as filed for example.

The solution to this problem, namely the provision of the tripeptide YSV, cannot be considered to be obvious in view of D1. D1 does not teach that the tripeptides therein can have these beneficial technical effects, as described in further detail below, and therefore cannot possibly motivate the skilled person to substitute YSL in the manner alleged by the Examiner to arrive at the present invention. Furthermore, to arrive at the peptide YSV starting from the disclosure of 30 peptides in D1 would require a large amount of experimental work for the skilled person, which is also indicative of an inventive step.

**I. D1 does not teach or suggest YSV as a pharmaceutical composition or the use thereof.**

Claims 4 to 8 are directed to a pharmaceutical composition comprising a tripeptide consisting of YSV and a method of using the same.

D1 teaches 30 biologically active peptides with various lengths, which include a tripeptide YSL. D1 does not disclose the tripeptide YSV, nor mention the use of the tripeptide YSV for any therapeutic effects. Nor does D1 suggest, or provide a motivation to use YSV for any therapeutic purpose. Therefore, D1 cannot deprive the present invention of an inventive step.

**II. Unexpected technical effects**

The present invention YSV possesses unexpected properties, being pharmacologically divergent and a surprising improvement over D1's YSL in its effects on leukemia, melanoma and liver cancers.

1. **Leukemia**

YSV inhibits human leukemia K562 cells both *in vitro* and *in vivo* (see WO 2005/00874, page 18, Table 3). On the contrary, YSL does not prolong the survival of mice with transplanted L1210 leukemia (see D1 Table IV.6 at page 50: only CMS 019 and CMS 035 have such an activity, but CMS 024, i.e., YSL, does not).

2. **Melanoma**

YSV inhibits the growth of transplanted melanoma in mice (see WO 2005/00874, page 22, Table 1). On the contrary, YSL does not prolong the survival of mice inoculated with melanoma (see

D1 section 8 at page 50: only CMS008 and CMS 016 have such an effect, but CMS 024, i.e., YSL, does not).

3. **Liver Cancer**

YSV is the most potent among the three tripeptides YSL, YSV, and YSF, in inhibiting the growth of human BEL 7402 hepatoma xenograft in mice. The efficacies of these three tripeptides are 36.7%, 64% and 20.1%, respectively (statistical significance, P<0.05). (See the comparative data below).

Table 1. Effects of YSV, YSL, and YSF on the growth of human BEL7402 hepatoma xenograft in nude mice

| Group | Dosage (/kg/day) | Animal | | Tumor weight | Tumor growth inhibition index (%) |
|---|---|---|---|---|---|
| | | Initial | end | | |
| YSL | 160μg | 10 | 10 | 0.878±0.499*$ | 36.7 |
| YSV | 160μg | 10 | 10 | 0.499±0.177*#$ | 64.0 |
| YSF | 160μg | 10 | 10 | 1.107±0.336* | 20.1 |
| Cyclophosphamide | 20mg | 10 | 8 | 0.191±0.049* | 86.2 |
| Saline | 0.2ml/day | 10 | 10 | 1.386±0.682 | - |

*: Comparing to saline group P<0.05

#: Comparing to YSL group P<0.05

$: Comparing to YSF group P<0.05

**III. Lack of motivation and reasonable expectation of success**

Furthermore, applicant must disagree with the Examiner's analysis of how the present invention may be derived starting from D1. To refer to a selection is perhaps misleading since D1 does not disclose or even emcompass YSV within its scope. However, in order for the skilled person to be motivated to arrive at the present invention, stating from D1, he must be motivated to search through over eight thousands six hundred and forty (8640) possible conservative substitutions to obtain the tripeptide YSV starting from D1. It is submitted that the Examiner erred in the scientific analysis regarding the number of possible substitutions and also failed to acknowledge the

apparent lack of motivation to modify YSL in the manner required to arrive at YSV for the reasons as follows.

D1 discloses 30 biologically active peptides and YSL is one of the 30 peptides. D1 teaches conservative replacement of one amino acid for another within the same functional class. Whilst D1 to replacement of non-polar residues, this is done merely as an example, and does not direct the skilled person to substitute these residues in preference to any other residues in the peptide. The wording of page 56, lines 37-42 confirms this because conservative changes are described generally before then referring to substitution of non-polar residues by way of example only. Thus, the skilled person is not motivated by D1 to firstly select YSL for modification and secondly to substitute only the final residue for Valine.

There are 6 amino acids within Tyrosine's function class (Amino acids with uncharged but polar side chains are Ser, Thr, Tyr, Asp, Glu, Cys) and 8 amino acids within Leucine's functional class (Amino acids with aliphatic hydrophobic side chains are Ala, Val, Leu, Ile, Met, Pro, Phe, and Trp). The selection of YSL from D1's 30 biologically active peptides in combination with the selection of Valine from 8 amino acids with hydrophobic side chains plus every possible replacement of Tyrosine's and Serine with amino acid residues within the same functional class does, it is submitted, require an inventive contribution starting from D1 as closest prior art, especially in view of the surprising technical advantages obtained from the resultant tripeptide.

First, the chance of selecting YSL from D1's 30 peptides is 1/30. To not include this factor is to view the present invention with hindsight, which of course must be avoided. Nothing in D1 teaches the skilled person to choose YSL as the sole peptide for further analysis. Second, there are 6*6*8, i.e., 288, possible number of replacements of YSL with amino acids within the same functional class. YSL has 3 amino acids, each of which may be replaced according to D1's teaching. The possible replacements for Y, S and L are 6, 6 and 8, respectively. Thus, the total number of possible combinations is 6*6*8, i.e., 288. Thus, if we take into account the possible substitutes of other peptides in D1 (such as Tyr Ser Phe and Ala Ala Phe), the possibility of obtaining YSV from D's 30 peptides is, in fact, less than 1/8640 (288*30=8640).

Considering there are thousands of possible replacements, it would be highly unlikely for the skilled person to design, make and realize all of the possible replacements, since such a research program would represent a large burden for the skilled person. Nor is it likely that the skilled person would be motivated to conduct pharmacological screening on the thousands of

possible peptide substituents. To select among thousands of possibilities and successfully achieve the present invention, the tripeptide YSV with its associated benefits in terms of treatment of leukaemia, melanoma and liver cancer, it is submitted that Applicants have contributed the required inventive step. Further, the pharmacological effects of YSV in leukemia, melanoma and liver cancers are surprising and outstanding and totally unexpected. None of this would have been obvious to one of skill in the art at the priority date of the present application. Therefore, the claimed invention necessarily involves an inventive step and the Examiner is respectfully requested to withdraw the Inventive step objection raised in respect of claims 4 to 8.

**Examiner's further objections**

**1. Claims 1, 4, 6, 8, 9 and 13-19**

The Examiner has raised a support objection against former claims 1, 4, 6, 8, 9 and 13-19, because these claims relate to the tripeptide YSV without specifying the amino acids being of the L-isomer form.

Claims 4, 6, 8, 13-16 and 19 have now been cancelled. Therefore, any issue related to these claims is moot. Applicants, however, respectfully disagree with the Examiner's remarks on this point concerning Claims 1, 9 and 18. Firstly, the specification refers throughout to the tripeptide YSV without any limitation to the L-form. Secondly, the Examiner's attention is drawn to page 11, lines 7-10 of the specification, which discloses that D-form amino acids forming a YSV tripeptide are contemplated and may have similar biological activities to L-form. Accordingly, the claims are considered to be adequately supported by the description.

However, for the sake of expediting prosecution of the application, all claims now specify that the [illegible] in the L-form.

**2. Claims 5 and 6**

The Examiner remarks that Claims 5 and 6 lack clarity due to being directed to the same subject matter. Claim 6 has now been cancelled.

**3. Claim 7**

The Examiner suggested deletion of former [illegible] 7 because it appears to be broader than claim 6, on which it depends. Both claims 6 and 7 now been deleted.

**4. Claim 19**

The Examiner raised an objection against former claim 19, alleging that the term "enhancement molecule" lacks meaning. While Applicants respectfully disagree, claim 19 has now been cancelled to expedite prosecution of the application.

**5. Industrial applicability**

The Examiner noted that claims 9 to 12 are directed to a method of treatment of the human or animal body. The Examiner pointed out that no unified criteria exist and the patentability can be dependent upon the formulation of the claims.

Claim 9 has now been renumbered as claim 4 and amended to specify the human disease being "selected from the group consisting of a condition which can be reduced by stimulating T lymphocyte transformation and a cell proliferative disorder." Claim 11, as amended and renumbered as claim 5, depends on claim 4 and claim 12, new claim 6, depends on claim 5. It is stated that the claims on file are considered to be industrially applicable in at least some PCT contracting states (such as the United States for example). For those PCT contracting states (such as the European Patent Office) where methods of treatment of the human or animal body are not considered to be patentable, applicant will amend the claims into the appropriate format during the prosecution before the relevant national/regional office.

**Conclusion**

In view of the above remarks, it is respectfully submitted that the claims are now in condition for the issuance of a favorable International Preliminary Examination Report.

Yours faithfully,

BALDOCK; Sharon Claire
Authorised Representative

Enclosure
632303; MPS; MPS

## Claims

1. An isolated or purified peptide consisting of the tripeptide L-Tyrosyl-L-Seryl-L-Valine.

2. The peptide of Claim 1 wherein said peptide has an activity selected from the group consisting of modulation of an immune response, stimulation of T lymphocyte transformation, modulation of a cell proliferative disorder, modulation of the growth of a cancer, modulation of the growth of a liver cancer, modulation of the growth of leukemia cells, modulation of the growth of a cervical cancer, modulation of the growth of a lung cancer and the modulation of the growth of a melanoma.

3. A pharmaceutical composition comprising a polypeptide consisting of the tripeptide L-Tyrosyl-L-Seryl-L-Valine.

4 A method of reducing the condition of a human disease comprisi administering a pharmaceutically effective dose of a polypeptide consisting of t tripeptide L-Tyrosyl-L-Seryl-L-Valine to a human, wherein said human disease is selected from the group consisting of a condition that can be reduced by stimulating T lymphocyte transformation and a cell proliferative disorder.

5. The method of Claim 4, wherein said cell proliferative disorder is canc

6. The method of Claim 5, wherein said cancer is selected from the gr consisting of liver cancer, leukemia, lung cancer, melanoma and cervical cancer.

7. The method according to Claim 4, wherein said disease may be treated by modulation of immune system.

8. Use of a peptide consisting of the tripeptide L-Tyrosyl-L-Seryl-L-Valine as a nutritional supplement.

**Amendments to the Claims**

1. (Previously presented) An isolated or purified peptide consisting of the tripeptide L-Tyrosyl-L-Seryl-L-Valine.

2. (Previously presented) The peptide of Claim 1 wherein said peptid has an activity selected from the group consisting of modulation of an immun response, stimulation of T lymphocyte transformation, modulation of a cell proliferative disorder, modulation of the growth of a cancer, modulation of the growth of a liver cancer, modulation of the growth of leukemia cells, modulation of the growth of a cervical cancer, modulation of the growth of a lung cancer and the modulation of the growth of a melanoma.

~~6.~~3. (Cancelled) ~~A peptide according to Claim 1 . . . tripeptide L-Tyrosyl-L-seryl-L-valine.~~

4. (Cancelled) ~~A peptide according to any of the Claims 1-3 wherein said peptide is in a substantially pure form.~~

5. (Currently amended) A pharmaceutical composition comprising a polypeptide consisting of the tripeptide L-Tyrosyl-L-Sseryl-L-Vvaline.

6. (Cancelled) ~~A pharmaceutical composition comprising a polypeptide consisting of the tripeptide Tyrosyl-seryl-valine.~~

7. (Cancelled) ~~A pharmaceutical composition according to Claim 6 comprising the tripeptide L-Tyrosyl-L-seryl-L-valine.~~

8. (Cancelled) ~~A method of making a pharmaceutical composition comprising providing the tripeptide Tyrosyl-seryl-valine and mixing said tripeptide with a pharmaceutically acceptable carrier.~~

9. (Currently amended) A method of reducing the condition ~~effects~~ of a human disease comprising administering a pharmaceutically effective dose of a polypeptide consisting of the tripeptide L-Tyrosyl-L-Seryl-L-Valine ~~seryl-valine~~ to a human, wherein said human disease is selected from the group consisting of a condition that can be reduced by stimulating T lymphocyte transformation and a cell proliferative disorder.

10. (Cancelled) ~~The method of Claim 9, wherein said human suffers from a disease selected from the group consisting of a condition whose effects can be reduced by stimulating T lymphocyte transformation and a cell proliferative disorder.~~

11. (Currently amended) The method of Claim 4910, wherein said cell proliferative disorder is cancer.

12. The method of Claim 511, wherein said cancer is selected from the group consisting of liver cancer, leukemia, lung cancer, melanoma and cervical cancer.

13. (Cancelled) ~~A tripeptide consisting of Tyrosyl seryl valine for use in the treatment of a disorder.~~

14. (Cancelled) ~~The use according to Claim 13, wherein said disorder is a cell proliferative disorder.~~

15. (Cancelled) ~~The use according to Claim 14, wherein said cell proliferative disorder is cancer.~~

16. (Cancelled) ~~The use according to Claim 15, wherein said cancer is selected from the group consisting of liver cancer, leukemia, lung cancer, melanoma and cervical cancer.~~

17. (Currently amended) The method ~~use~~ according to Claim 49 ~~13~~, wherein said disease ~~disorder~~ may be treated by modulation of ~~the~~ immune system.

18. (Currently amended) Use ~~The use~~ of a peptide consisting of the tripeptide L-Tyrosyl-L-Sseryl-L-Vvaline as a nutritional supplement~~, wherein the amino acid residues are all in the L isomer form~~.

~~19.~~(Cancelled) ~~A molecule comprising an enhanced derivative of the tripeptide Tyrosyl seryl valine, said enhanced derivative comprising an enhancement molecule operably linked to the tripeptide Tyrosyl seryl valine, said enhancement molecule enhancing the therapeutic effectiveness of said tripeptide.~~